CLAIMS

1. Process for obtaining new eukaryotic strains, preferably new yeast strains, and even more preferentially strains of *Saccharomyces cerevisiae* conserving stress resistance in the presence of fermentable sugars such as glucose, comprising the following steps :

a classic mutagenic treatment is carried out on the cells of a starting strain,

the cells having undergone the said mutagenic treatment are cultured so as to obtain a stationary phase,

the said cells in stationary phase are incubated in the presence of at least one fermentable sugar selected from the group comprising glucose, maltose, and sucrose, this sugar being present in a quantity such that the cells enter an active metabolic state (fermentation and/or growth) of this sugar,

said cells in active metabolic state are subjected to one or several stresses leading to a mortality rate of at least 99% with respect to the starting population,

the surviving cells are isolated and

those of the surviving cells which respond to the following criteria which characterize the fil phenotype are selected, i.e. :

- a growth, evaluated by production or production yield of biomass over sugar in a given time or by a growth rate, under identical culture conditions, at least equal to 80% of the starting strain, and preferably at least equal to 90% of the starting strain,
- a CO_2 release, or a metabolite production, in identical conditions, at least equal to 80%, and preferably at least equal to 90% of the starting strain,
- a stress resistance, corresponding to a survival rate at least 2 times higher, preferably at least 3 times higher, more preferentially at least 5 times higher, and even more preferentially at least 10 times higher than the survival rate of the starting strain, under identical phase conditions corresponding to a growth or active metabolism followed by a heat shock of at least 20 minutes at 52°C, or at least 1.5 times higher, preferably at least 2 times higher, more preferably at least 3 times higher, and even more preferentially at least 5 times higher than the survival rate of the starting

strain, under identical conditions of growth phase followed by freezing for a period of at least 24 hours at -20°C or at a lower temperature,

- maintenance of these properties after repeated cultures on non selective medium, such as YPD medium, so as to verify that the fil phenotype obtained by the mutation is perfectly stable and permanent.

2. Process according to claim 1, wherein it is checked that any useful secondary property has not been lost and that any hampering property has not appeared.

3. Process according to claim 1, wherein the starting strain is an industrial strain.

4. Process according to claim 3, wherein an industrial fil mutant carrying several mutations is obtained and wherein :

- the segregants issued from this industrial mutant are crossed with a laboratory haploid strain to select the segregant issued from this industrial mutant giving to the polyploids obtained with the laboratory strain an improvement in the required properties;
- the segregants thus selected are crossed one with the other;
- the polyploids obtained are selected according to the criteria of fil phenotypes defined in claim 1.

5. Process according to claim 1, wherein the selected fil strains preferably have the property of conserving, in growth and/or fermentation phase on fermentable sugars, at least 50%, preferably at least 60%, more preferentially at least 70%, and even more preferentially at least 80% of their survival rate with respect to the survival rate in stationary phase measured under the same conditions after a heat or freeze shock.

6. Process according to claim 1, wherein the cells obtained after mutagenesis treatment are introduced into pieces of dough subjected to at least 100 cycles of freezing/thawing after a first fermentation of the dough of 30 minutes at 30°C.

7. New industrial eukaryotic strain, preferably of yeast and still more preferably belonging to the *Saccharomyces* genus having the fil phenotype, obtainable by the process according to claim 1.

8. New industrial yeast strain, preferably belonging to the *Saccharomyces* genus and still more preferably belonging to the *Saccharomyces cerevisiae* species having the fil phenotype, obtainable by the process according to claim 2.

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9. New strain according to claim 7, belonging to *Saccharomyces cerevisiae* species.

10. New yeast strain according to claim 7 having a survival rate, in growth phase on fermentable sugars, of at least 50%, preferably at least 60%, more preferably at least 70% and still more preferably at least 75%, after a heat treatment of 20 minutes at 52°C, , the growth phase being defined as a reculturing on fermentable sugar (glucose) of 10 minutes at 30°C after stationary phase.

11. New yeast strain according to claim 8 having a survival rate, in growth phase on fermentable sugars, of at least 50%, preferably at least 60%, more preferably at least 70% and still more preferably at least 75%, after a heat treatment of 20 minutes at 52°C, , the growth phase being defined as a reculturing on fermentable sugar (glucose) of 10 minutes at 30°C after stationary phase.

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12. New industrial yeast according to claim 7 whose stability to freezing in lumps of dough incubated 60 minutes at 30°C before freezing and containing 20 g of flour, 15 g of water, 1 g of sucrose, 0.405 g of NaCl, 0.06 g of $(NH_4)_2SO_4$ and 160 mg of dry matter of the considered strain, defined by the ratio between the release of CO_2 at 30°C after 1 month or 30 days of conservation at -20°C and the release of CO_2 at 30°C after 1 day of conservation at -20°C, is at least equal to 80%, preferably at least equal to 85% and more preferably at least equal to 90%.

13. New industrial yeast strain according to claim 8, whose stability to freezing in lumps of dough incubated 30 minutes at 30°C before freezing and containing 20 g of flour, 15 g of water, 0.405 g of NaCl, 0.06 g of $(NH_4)_2SO_4$ and 160 mg of dry matter of the considered strain, measured by the ratio between the release of CO_2 at 30°C after 1 month or 30 daysof conservation at -20°C and the release of CO_2 at 30°C after 1 day of conservation at -20°C, is at least higher than 80%, preferably at lest higher than 85% and more preferably at least higher than 90%.

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14. New yeast strain according to claim 7, whose loss of released gas after drying of the biomass harvested in a phase close to exponential growth phase is at most equal to 67%, preferably at most equal to 50% of the loss of released gas after drying of yeasts obtained using the corresponding starting strain or a control strain having the same characteristics.

15. New strain PVD1150 = M5 *fil1* deposited at C.N.C.M. under the n° I-2031 and the n° I-2203.

16. New strain KL1 = W303 *fil2* deposited at C.N.C.M. under the n° I-2032.

17. New strain FD51 = HL816 *fil300* deposited at C.N.C.M. under the n° I-2033.

18. New strain FDH16-22 = HL822 *fil300* deposited at C.N.C.M. under the n° I-2034.

19. New strain AT25 = S47 *fil400* deposited at C.N.C.M. under the n° I-2035.

20. New strain AT28 = S47 *fil500* deposited at C.N.C.M. under the n° I-2036.

21. New strain AT251 deposited at C.N.C.M. under the n° I-

22. New strain AT252 deposited at C.N.C.M. under the n° I-

23. New strain AT254 deposited at C.N.C.M. under the n° I-

24. New strains belonging to the same kind than strains AT25 and AT28.

25. New strains belonging to the same kind than strains AT251, AT252 and AT24.

26. Mutant gene obtainable by isolation from one of the mutant strains obtainable by the process according to claim 1.

27. Gene according to claim 26, conferring the fil phenotype to one of the strains according to claim 7.

28. Gene *CDC35* = *CYR1* carrying a mutation conferring the fil phenotype.

29. Gene according to claim 28, wherein the mutation is a change of a G base into an A base in the region of the gene *CDC35/CYR1* coding for the catalytic site of the enzyme, equivalent to a change of an acidic amino acid (glutamic acid) into a basic amino acid (lysine) at position 1682 of the protein.

30. Gene *GPR1* carrying a mutation conferring the fil phenotype.

31. Gene according to claim 30 carrying the mutation of the KL1=W303 fil 2 strain.

32. Gene having properties similar or equivalent to those of one of the genes according to claim 27, i.e. gene carrying a mutation conferring the fil phenotype, and belonging to the family of genes:

- coding for proteins having a function comparable to that of a protein coded by one of the genes according to claim 27 in yeast or another eukaryote,

- coding for proteins associated with the protein coded by one of the genes according to claim 27,
- coding for proteins having similar sequences, i.e. at least 60% homology, preferably at least 70% homology and still more preferably at least 80% homology with the protein coded by one of the genes according to claim 27.

33. Gene according to claim 32, coding for a protein associated with the protein coded by the *GPR1* gene according to claim 30 wherein the said gene may be a *GPA* gene as the *GPA2* gene of yeast carrying a mutation which confers the fil phenotype.

34. Eukaryotic strain transformed in a manner so that at least certain of the alleles of the gene according to claim 26 or genes analogous to these genes carry a mutation which confers the fil phenotype.

35. Yeast strain transformed in a manner so that at least certain of the alleles of the gene according to claim 26 carry a mutation which confers the fil phenotype.

36. Process for obtaining baker's yeast intended for frozen doughs comprising the use of a strain according to claim 7.

37. Process for obtaining baker's yeast intended for frozen doughs comprising the use of a strain according to claim 8.

38. Process for obtaining dry baker's yeast comprising the use of a strain according to claim 7.

39. Process for obtaining brewery yeast comprising the use of a strain according to claim 8.

40. Process for obtaining brewery yeast comprising the use of a strain according to claim 7.

41. Process for obtaining yeasts intended for the production of alcohol comprising the use of a strain according to claim 7.

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SUB A6

SUB A7

ADD 7 A8